Microfluidic Separation and Measurement of Biomolecules Using Gradient Elution Moving Boundary Electrophoresis

As part of an ongoing effort to develop new microfluidics-based technologies for chemical and biochemical analyses, NIST scientists invented a new technology called gradient elution moving boundary electrophoresis (GEMBE). GEMBE has allowed for the development of higher throughput, parallel biochemical analysis devices without requiring a significant increase in device size as seen with other techniques. This new technology is expected to enable better, faster and cheaper measurements of chemicals and biomolecules for drug discovery, homeland security and medical diagnostics.

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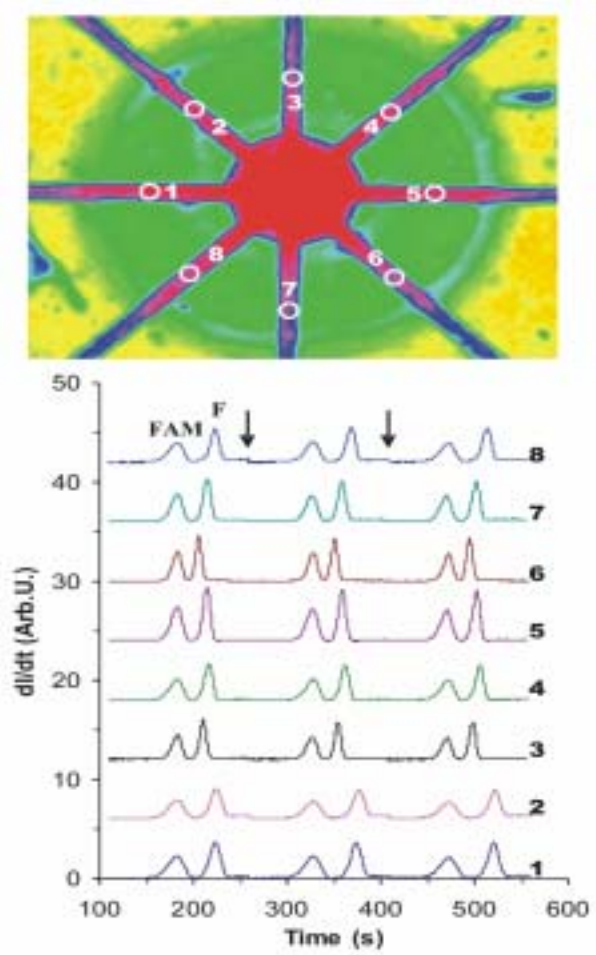
To realize the full potential of miniature microfluidic devices for totally integrated and portable measurement of chemicals and biomolecules, separation methods that can be performed on very short length scales are required. NIST's new GEMBE is a novel method for performing electrophoretic separations, enabling quantitative analyses in microchannels less than one centimeter in length.

GEMBE utilizes the electrophoretic migration of chemical species in combination with variable hydrodynamic bulk counter-flow of the solution through a separation capillary or microfluidic channel. Continuous sample introduction eliminates the need for a sample injection mechanism. Only the analytes with an electrophoretic velocity greater than the counter-flow velocity enter the separation channel. The counter-flow velocity is varied over time so that each analyte is brought into the separation column at different times, allowing for high-resolution separations in very short channels. The new variable of bulk flow acceleration affords a new selectivity parameter to electrophoresis analogous to gradient elution compositions in chromatography. Because it does not require extra channels or access ports to form an injection zone and because separations can be performed in very short channels, GEMBE separations can be implemented in much smaller areas on a microfluidic chip as compared to conventional capillary electrophoresis.

NIST's new separation method allows for reduced size requirements for portable microfluidic devices. Higher analysis element density will allow for more efficient measurements on smaller devices.



The figure shows an eight channel multiplexed microfluidic GEMBE separation. (Top) False color fluorescence image of detection region of microchip; the 7.1 mm long channels contain fluorescein and carboxyfluorescein (FAM). The common control port and the eight radially arranged channels are seen as red (blue at the outer edges of the image). The eight detection points are shown as the small white circles. (Bottom) Derivative intensity (dI/dt) plots for parallel separation of fluorescein (F) and FAM (500 nmol/L of each). Sample was loaded into each of the eight sample reservoirs; traces relate to each channel with three sequential runs (delineated by arrows).

GEMBE separations of small dye molecules, amino acids, DNA, and immunoassay products have been demonstrated. A low-cost polymeric eight-channel multiplexed microfluidic device was also fabricated to demonstrate the reduced area requirements of GEMBE; the device was less than

one square inch in area and required only n+1 fluidic access ports per n analyses (in this instance nine ports for eight analyses). Parallel separations of fluorescein and carboxyfluorescein yielded less than 3 % relative standard deviation (RSD) in inter-channel migration times and less than 5 % RSD in both peak and height measurements. The device was also used to generate a calibration curve for a homogeneous insulin immunoassay using each of the eight channels as a calibration point with less than 5 % RSD at each point with replicate analyses.

Future Plans: Research using GEMBE is on going as the technique's fundamental figures of merit are explored, and the method is optimized. The potential to measure complex samples, such as found in biology, is being investigated, as the counter flow allows for the exclusion of separation degrading matrix interferants.

Publications:

Shackman, J.G.; Ross, D. **"Electrophoretic Separations in small spaces: Gradient Elution Moving-Boundary Electrophoresis (GEMBE)"**, In *Proceedings of μTAS 2006 Conference*, Kitamori, T.; Fujita, H.; Hasebe S. Eds.; Society for Chemistry and Micro-Nano Systems, Tokyo, 2006; pp. 912-914.

Shackman, J.G.; Munson, M.S.; Ross, D. **"Gradient Elution Moving Boundary Electrophoresis (GEMBE) for High-Throughput Multiplexed Microfluidic Devices"**, *Analytical Chemistry* In Press 2006.

Shackman J.G.; Ross, D. **"Gradient Elution Moving Boundary Electrophoresis (GEMBE)"**, Recommended for U.S. Patent by NIST Patent Review Committee, Nov. 2006 (Disclosure 06-011).